Simultaneous nitrification and denitrification using a novel up-flow bio-electrochemical reactor

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\section*{Abstract}

Nitrogen removal is a problem in the field of water treatment, especially in the presence of sulfate. Conventional nitrification and denitrification are usually carried out in two separate reactors. In addition, the effect of sulfate on hydrogenotrophic denitrification is not clear. In this study, simultaneous nitrification and denitrification (SND) for nitrogen removal from water was conducted using a single novel up-flow bio-electrochemical reactor (UBER). The influence of dissolved oxygen (DO) on nitrogen removal was investigated. When influent DO was 7.0–8.0 mg L\textsuperscript{-1}, a heterotrophic nitrification zone (with DO 3.2–5.5 mg L\textsuperscript{-1}) and a hydrogenotrophic denitrification zone (with DO 1.6–4.2 mg L\textsuperscript{-1}) were obtained within the reactor, and the removal rates of NH\textsubscript{4}\textsuperscript{+}-N and TN reached more than 90\%. The distribution of DO inside developing biofilms was measured using microelectrodes. When DO in the hydrogenotrophic denitrification zone was 2.9 mg L\textsuperscript{-1}, DO inside the biofilm was just 0.5 mg L\textsuperscript{-1}. The effect of sulfate on hydrogenotrophic denitrification was studied by regulating the S/N ratio of influent water. Simultaneous removal of nitrate and sulfate can be achieved at low S/N, and the removal rates of nitrate and sulfate were ~80\%. With increasing S/N ratio, sulfide produced by sulfate reduction inhibited both denitrification and further sulfate reduction.

\textbf{Keywords:} Nitrification and denitrification; Bio-electrochemical reactor; Biofilm; Sulfate

1. Introduction

Nitrogenous contaminants such as nitrate and ammonia can promote eutrophication, causing deterioration of water quality and posing potential hazards to human or animal health [1]. Therefore, different technologies such as reverse osmosis, chemical denitrification and biological denitrification have been developed to remove nitrogenous contaminants from water bodies [2]. Simultaneous nitrification and denitrification (SND) is one of the most widely accepted biological solutions for removing nitrogen from high ionic strength nitrogenous wastewaters [3]. SND is highly effective at removing nitrogen compounds [4,5] because it uses small reaction volumes, has short reaction times and low energy consumption [6,7]. It is estimated that the SND process utilizes 22\%–40\% less carbon and reduces sludge yield by 30\% compared with conventional nitrification and denitrification systems [8]. Through the SND process, oxygen and NO\textsubscript{3}–N can fully be utilized as the alternate electron acceptors, which results in low DO [9,10]. Additionally, SND can be accomplished at neutral pH because it is self-buffering, with alkalinity produced during denitrification consumed during nitrification. Robertson et al. [11] reported that the experimental conditions for SND
were difficult to control in one reactor. Consequently, it is necessary to develop a novel reactor for SND to ensure different microbial communities are distributed effectively, and do not change with changing influent load.

The “bio-electrochemical reactor” system is a novel method for water and wastewater denitrification that improves biological denitrification by immobilizing autohydrogenotrophic bacteria directly on the surface of a cathode to provide easy access to NO_3^- and H_2 as the electron acceptor and electron donor, respectively [12]. Eq. (1) shows the general reaction leading to autohydrogenotrophic denitrification in aqueous solution. Ghafari et al. [13] demonstrated co-existence of both aerobic and anoxic zones in a single up-flow bio-electrochemical reactor (UBER), which had a high chemical oxygen demand (COD) and efficient nitrogen removal.

\[
2\text{NO}_3^- + 5\text{H}_2 \rightarrow 4\text{H}_2\text{O} + \text{N}_2 + 2\text{OH}^- \tag{1}
\]

Another limiting factor on N removal treatment systems is sulfate, which is common in natural water bodies and wastewaters. Under anaerobic or anoxic conditions, nitrate and sulfate can be reduced to nitrogen and sulfide by denitrifying bacteria and sulfate reducing bacteria, respectively. Nitrate reduction is thermodynamically more favourable than sulfate reduction [14]. Chen et al. [15] found that the degree of SO_4^2- reduction steadily decreased with higher influent NO_3^- concentration. Conversely, the end product of sulfate reduction, sulfide, is harmful to microorganisms at high concentration and has the potential to inhibit N removal processes and prevent further sulfate reduction. The relationship between nitrate and sulfate in low DO environments therefore needs further study.

The goal of this study was (1) to design a novel reactor which combined heterotrophic nitrification and hydrogenotrophic denitrification for SND (2) to investigate nitrogen removal efficiency and DO distribution in biofilms in the reactor and (3) to explore the effect of sulfate on hydrogenotrophic denitrification.

2. Materials and methods

2.1. Experimental apparatus

A schematic of the lab-scale novel UBER used in the study is shown in Fig. 1. The new UBER for SND was divided into two functional units, a lower heterotrophic nitrification zone and an upper hydrogenotrophic denitrification zone, to ensure different microbial communities were distributed effectively. The apparatus for experiments on the effect of sulfate has the same volume and arrangement of experimental materials but without the heterotrophic nitrification zone.

The UBER was built using a 2 L Plexiglass cylindrical column (inside diameter of 9.2 cm, height 35 cm), sealed at the top. A stainless steel wire mesh was installed at the middle of the reactor as a cathode and a carbon rod (8.8 cm long) was installed at the top of the reactor as the anode. An adjustable power supply (APS3005D, Shenzhen, China) was applied to provide direct current. One inlet port was installed at the bottom of the cylindrical column, and one outlet port was installed 27 cm from the bottom, leaving a 3 cm head space. Sampling points were installed every 5 cm from the bottom. Sampling tap 1 and tap 2 were installed 25 cm and 10 cm from the bottom, respectively. The reactor was filled with carbon granules (in the size range of 1–2 cm) which were washed with deionized water four times prior to use. To provide a sticky surface for microorganisms on the carbon granules, they were saturated and boiled in 2% agar solution. The total volume of carbon granules was 1 L, accounting for 50% of the reactor’s capacity. The reactor was covered with aluminium foil to exclude light and prevent algal growth.

2.2. Synthetic influent and sludge

Based on the water quality that is characteristic of local polluted rivers, reservoirs and groundwater [16], synthetic wastewater for this work was prepared with a low C/N ratio. The composition of synthetic wastewater for the SND experiments comprised glucose (0.6 g L^-1), NH_4Cl (0.23 g L^-1), KH_2PO_4 (0.013 g L^-1), MgSO_4·7H_2O (0.02 g L^-1), CaCl_2·2H_2O (0.001 g L^-1), FeSO_4·7H_2O (0.001 g L^-1), NaHCO_3 (0.252 g L^-1) and 1 mL trace solution. The components of the trace solution were ZnSO_4·7H_2O (100 mg L^-1), MnCl_2·4H_2O (30 mg L^-1), H_3BO_3 (300 mg L^-1), CoCl_2·6H_2O (200 mg L^-1), CuCl_2·2H_2O (10 mg L^-1), NiCl_2·2H_2O (10 mg L^-1), Na,MnO_2·2H_2O (30 mg L^-1) and Na_2SeO_3 (30 mg L^-1). Oxygen (O_2) was added from a gas cylinder to adjust the DO of the influent on demand. Aerobic and anaerobic sludge were obtained from a secondary sedimentation tank and an anaerobic digester.
tank in the Xin'anhe Municipal Wastewater Treatment Plant in Yantai, China. Aerobic and anaerobic sludge were aerated with oxygen and bubbled with nitrogen, respectively, for 24 h. The two kinds of activated sludge were mixed in equal volumes prior to pouring (1 L) into the reactor.

The simulated wastewater for the sulfate effect experiments comprised NaHCO₃ (0.252 g L⁻¹), MgSO₄·7H₂O (0.34 g L⁻¹), FeCl₃ (0.1 g L⁻¹), KH₂PO₄ (0.027 g L⁻¹), CaCl₂ (0.3 g L⁻¹), 1 mL trace solution I and 1 mL trace solution II. The components in trace solution I were EDTA (5 g L⁻¹), FeSO₄ (5 g L⁻¹). The components in trace solution II were EDTA (15 g L⁻¹), H₂BO₃ (0.014 g L⁻¹), MnCl₂·4H₂O (0.99 g L⁻¹), CuSO₄·5H₂O (0.25 g L⁻¹), CoCl₂·6H₂O (0.24 g L⁻¹), ZnSO₄·7H₂O (0.43 g L⁻¹), NiCl₂·6H₂O (0.19 g L⁻¹), Na₂MoO₄·2H₂O (0.22 g L⁻¹) and Na₂SeO₃·10H₂O (0.21 g L⁻¹). The concentrations of NaNO₃ and Na₂S were added as required for the experiment. The simulated wastewater was purged with nitrogen for 1 h to remove residual oxygen. Anaerobic sludge was bubbled with nitrogen for 24 h before pouring (1 L) into the reactor.

2.3. Experimental conditions

The removal rates of NH₄⁺-N and total nitrogen (TN) in the reactor were investigated under different conditions. At the beginning of the experiment, the pH of the synthetic wastewater was adjusted to 7.5 using NaHCO₃. The temperature was controlled at 30°C ± 2°C to accelerate the reaction rate and shorten the experimental period. The bio-electrochemical reactor was operated with a feed of 200 mL/h synthetic wastewater (hydraulic retention time = 10 h). DO concentration in the bulk solution inside the reactor was set by adjusting inflow at different phases. The UBER experiment lasted 95 d and was divided into four phases: days 1–30, 31–50, 51–70 and 71–95 (Table 1). These phase divisions ensured that the biofilm had enough time to mature and stabilize. In phase 1, the influent DO was 60 mg L⁻¹ in phase 2, 7 mg L⁻¹ in phase 3, and to 8 mg L⁻¹ in phase 4 (Table 1).

The effect of sulfate on hydrogenotrophic denitrification performance in the reactor was studied by regulating the influent S/N. Three experiments were conducted with S/N ratios of 1:2 (SO₄²⁻–S: 25 mg L⁻¹, NO₃⁻–N: 50 mg L⁻¹), 1:1 (SO₄²⁻–S: 50 mg L⁻¹, NO₃⁻–N: 50 mg L⁻¹) and 2:1 (SO₄²⁻–S: 50 mg L⁻¹, NO₃⁻–N: 25 mg L⁻¹), respectively. The experiments were carried out at 30°C ± 2°C, 10 mA electric current and 6 h of hydraulic retention time until the effluent parameters were stable.

2.4. Sampling and analysis

Samples were collected from the sampling taps. pH, temperature (T) and DO were measured immediately using a pH meter (PSH-3C, China), thermometer, and oxygen microelectrode (PRO 3.0, Unisense, Denmark). The COD of the effluent was measured using the potassium dichromate method. Then, remaining water samples were filtered using 0.2 μm syringe filters prior to analysis for NH₄⁺–N, NO₃⁻–N and NO₂⁻–N using an Autoanalyzer III (Seal, Germany) with an analytical precision of 0.5‰ unit. SO₄²⁻–S and sulfide were analyzed by an ion chromatograph (Dionex ICS3000, USA) and iodometric titration method [17], respectively. TN was detected using an UV spectrophotometry meter (TU-1950, Persee, Beijing, China). The DO distribution in the biofilm (adhered to the carbon granule surface) with depth was measured using a miniaturized Clark-type oxygen sensor with a guard cathode (DO microsensor, Unisense Microsensor, Denmark). A Micro Profiling System (Unisense) was used to control the penetration distance and acquire data.

3. Results and discussion

3.1. Start-up of the novel UBER

DO level, electric current and hydraulic retention time are three important factors in the nitrification and denitrification process. In this study, the novel UBER was operated for 95 d (phases 1–4) with different influent DO values (Table 1). During phase 1, high current (20 mA), high temperature (30°C), short hydraulic retention time (10 h) and 5.0 mg L⁻¹ DO were applied to supply sufficient substrates to support microbial activity (inoculated aerobic sludge and anaerobic sludge). The possible electrochemical reactions at the anode include the following:

\[ C + 2H₂O → CO₂ + 4H⁺ + 4e⁻ \quad (e^0 = 0.207 \text{ V}) \]  
\[ H₂O → \frac{1}{2}O₂ + 2H⁺ + 2e⁻ \quad (e^0 = 1.229 \text{ V}) \]

And the possible electrochemical reactions at the cathode are as follows:

\[ 2H⁺ + 2e⁻ → H₂ \quad (e^0 = 0.000 \text{ V}) \]  
\[ 2H₂O + 2e⁻ → H₂ + 2OH⁻ \quad (e^0 = -0.828 \text{ V}) \]

According to Reactions (2) and (3), CO₂ is formed prior to O₂ at the anode. This CO₂ could serve as pH buffer and inorganic carbon source. The hydrogen gas produced from the cathode serves as the electron donor for hydrogenotrophic denitrification.

Fig. 2 shows the variations in water quality between the lower and upper zone. In the first 2 d, the effluent...
The concentration of NH$_4^+$-N was a little higher than initial influent concentration (60 mg L$^{-1}$), which may be due to the death of bacteria which cannot adapt to the influent conditions. In the lower zone, NH$_4^+$-N and COD declined sharply while NO$_3^-$-N increased gradually and remained stable during the whole period. During phase 4, the steady concentrations of NH$_4^+$-N, NO$_3^-$-N and NO$_2^-$-N were 3.5, 1.5 and 24.1 mg L$^{-1}$, respectively. There were ~56.5 mg L$^{-1}$ N removed as NH$_4^+$-N and 25.6 mg L$^{-1}$ N produced as NO$_3^-$-N and NO$_2^-$-N. The removal rate of NH$_4^+$-N reached 96.5% at the end of phase 4 (Fig. 2c). These results indicate that nitrification occurred in the lower zone. This may include a variety of nitrification reactions, such as heterotrophic nitrification and autotrophic nitrification. In contrast chemolithotrophic nitrifiers, heterotrophic nitrifiers can use both inorganic and organic substrates for nitrification [18,19]. A high C/N ratio can stimulate the growth of heterotrophic bacteria and inhibit the activity of autotrophic nitrifiers [20]. In the presence of large amounts of organic matter, autotrophic nitrifying bacteria have less competition for oxygen and organic matter than aerobic heterotrophic bacteria, allowing the heterotrophs to become predominant.

In the upper zone effluent water, there were no significant variations in NH$_4^+$-N and NO$_3^-$-N between the upper zone effluent and the lower zone effluent, but the concentration of NO$_3^-$-N showed a distinct decline. This implied that denitrification mainly occurred in the upper zone. Both H$_2$ and organic matter can be used as electron donor for denitrification in the reactor. The maximum denitrification rate in the upper zone was 0.055 kg NO$_3^-$-N/ (m$^3$ d), and it was close to the similar bioelectrochemical denitrification reactor, indicating that hydrogenotrophic denitrification dominated in the upper zone.

In general, the hydrogenotrophic denitrification occurs at lower rates than heterotrophic denitrification owing to slower bacterial growth rates [2]. For example, Hamlin et al. [21] used four kinds of organics as carbon sources and the obtained maximum daily denitrification rate was 0.67–0.68 kg NO$_3^-$-N/(m$^3$ d), regardless of the carbon source. The average denitrification rate was 0.62 kg NO$_3^-$-N/(m$^3$ d) in the ethanol supported system [22], Sungur and Bose [23] achieved a denitrification rate of 0.027 kg NO$_3^-$-N/(m$^3$ d) in a fixed-bed hydrogenotrophic denitrification system. Park et al. [24] achieved a higher denitrification rate (0.077–1.68 kg NO$_3^-$-N/(m$^3$ d)) using a bio-electrochemical reactor.

After 30 d, concentrations of NH$_4^+$-N, NO$_3^-$-N and COD in the upper zone effluent reached 19.2, 8.6 and 22.3 mg L$^{-1}$, respectively (Fig. 2). Generally, stable water quality of the outlet and the color of biofilm can be used as indicators of the mature status of the biofilm. In this study, stable water quality and dark brown biofilm on the carriers (carbon granules) showed that the microbiological UBER systems had established after 30 d. In the lower zone, NO$_3^-$-N increased to 26.3 mg L$^{-1}$ at the end of phase 1 and remained at similar levels from phase 2 to phase 4. Meanwhile, NH$_4^+$-N decreased to ~3 mg L$^{-1}$ from phase 2 to phase 4, and the removal rate of COD reached 95.8% at the end of phase 4. In the upper zone, after phase 2, both of NO$_3^-$-N and NO$_2^-$-N were <5 mg L$^{-1}$, and NH$_4^+$-N and COD kept low levels (<5 and ~15 mg L$^{-1}$, respectively). These results demonstrate that heterotrophic nitrification and hydrogenotrophic denitrification was stable in...
the lower and upper zone, respectively. As shown in Fig. 2c, microbes maintained the ability to remove organic matter with more than 90% COD removal rate during the process of inoculation and acclimation (phase1). In the last phase, the removal rate of COD was up to 98%. The COD removal efficiency of the bio-electrochemical reactor was excellent.

3.2. Influence of DO on the nitrogen removal

During the experimental process, influent DO levels in influents were adjusted to 5, 6, 7 and 8 mg L\(^{-1}\) in phases 1, 2, 3 and 4, respectively. The relationship between DO and nitrogen removal is shown in Fig. 2 and 3. As shown in the lower heterotrophic nitrification section, the NH\(_4\)–N and TN removal rates in phase 2 were 83.3% and 37.5%, respectively, with 3.2 mg L\(^{-1}\) DO. In phase 3, DO increased to 4.8 mg L\(^{-1}\), and the removal rates of NH\(_4\)–N and TN gradually increased to 93.3% and 49.5%, respectively (Fig. 2c). In the upper hydrogenotrophic denitrification section, the removal rates of NH\(_4\)–N and TN reached 80% while the DO level was 1.7 mg L\(^{-1}\) at phase 2. In phase 3, NH\(_4\)–N and TN removal rates achieved 90% with 2.4 mg L\(^{-1}\) DO level (Fig. 2c).

In phase 4, the DO levels in bulk solution increased further to 5.5 and 4.2 mg L\(^{-1}\) in the heterotrophic nitrification and hydrogenotrophic denitrification zones, respectively, by increasing influent DO levels to 8.0 mg L\(^{-1}\). At this stage, the effluent quality parameters such as NH\(_4\)–N and NO\(_3\)–N remained stable (Fig. 2). Meanwhile, the TN removal rates of the reactor were kept stable (above 90%). This phenomenon indicated that the hydrogenotrophic denitrification was not restricted by relatively high DO level (4.2 mg L\(^{-1}\)). Deng et al. [25] had similar results, showing that the autotrophic denitrification process using hydrogen from Fe–C galvanic cells as an electron donor was not affected by DO. Li et al. [26] also had similar findings, with maximum nitrogen removal efficiency of 96.5% while the DO concentrations of influent and effluent were 7.95 and 6.74 mg L\(^{-1}\), respectively. As shown in Fig. 3, DO levels were well below the influent levels throughout. The decline of DO concentrations (about 1.3 mg L\(^{-1}\)) in the hydrogenotrophic denitrification zone between influent and effluent was likely due to consumption by aerobic denitrifiers [27]. The microbial community in the reactor needs to be studied.

3.3. Simultaneous nitrification and denitrification

At the end of the experiment (95 d, four phases), the concentrations of NH\(_4\)–N, NO\(_2\)–N, NO\(_3\)–N, TN and COD at different depths of the reactor were measured. As shown in Fig. 4, NH\(_4\)–N and COD abruptly decreased to the lowest value (close to zero) with depth. However, NO\(_3\)–N increased gradually in the heterotrophic nitrification zone (nitrification dominated the nitrogen removal process), then decreased in the hydrogenotrophic denitrification section (denitrification dominated the process); almost no NO\(_2\)–N accumulated in the whole process. In the heterotrophic nitrification zone, the concentration of NH\(_4\)–N decreased from 56 to 2.1 mg L\(^{-1}\) (Fig. 4) while both NO\(_3\)–N and NO\(_2\)–N increased, which proved that nitrification occurred. Meanwhile, the TN removal rate (above 50%) during phase 4 in Fig. 2c illustrates that significant denitrification took place in this point. As for the hydrogenotrophic denitrification section, NH\(_4\)–N and COD decreased gradually with the reactor height, which showed partial nitrification could occur in this section. NO\(_3\)–N went up to 10.6 mg L\(^{-1}\) first and then reduced to 2.1 mg L\(^{-1}\) (Fig. 4), moreover, there was similar variation trend in NO\(_2\)–N. This suggests both nitrification and denitrification could occur in the upper denitrification zone. These phenomena confirmed that SND had been achieved in the different parts of the reactor.

The transfer and consumption of DO in the biofilm serve important functions in nitrogen removal in the UBER system. Excessively high DO transfer resistance in the biofilm results in the aerobic layer being too thin and complicates ammonia oxidation. Conversely, excessively low DO transfer resistance makes the anaerobic layer too thin and slows down denitrification [28,29]. Determining the DO content in the biofilm is helpful for understanding the mechanism of nitrogen removal. The DO microdistributions

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*Fig. 3. Variations of DO in the heterotrophic nitrification zone and hydrogenotrophic denitrification zone.*

*Fig. 4. Water quality parameters at different depths of the reactor.*
(by microelectrode) in the nitrification and denitrification biofilms are shown in Fig. 5. In the heterotrophic nitrification zone, the thickness of biofilm at phase 1 was 500 μm and then increased with time. Consequently, the thickness of biofilm increased to 1,650 μm during phase 4. There was a similar pattern in the hydrogenotrophic denitrification zone, where the thickest biofilm was 1,100 μm at phase 4. The thickness of both biofilms increased with time, showing a continued growth of the microbial communities. It also can be seen that biofilm thicknesses in the heterotrophic nitrification section were thicker than those in the hydrogenotrophic denitrification section at the same phase. This result was in accordance with the fact that heterotrophic microorganisms have faster growth rates than autotrophic microbes. For the DO microdistribution in biofilms in the heterotrophic nitrification zone (Fig. 5a), the DO levels in the biofilm declined to approximately 1.1 mg L⁻¹ and then maintained a similar level, though the bulk DO values were different in different phases. Similar trends were shown in the hydrogenotrophic denitrification zone (Fig. 5b), where the DO levels in the biofilms continuously dropped to nearly 0.5 mg L⁻¹. The maximum DO in the upper and lower parts were 4.2 and 5.5 mg L⁻¹, respectively. DO in biofilms decreased with the depth of biofilms at all phases. Thus, nitrification occurred in the outer layer of the biofilms whereas denitrification can occur in the inner layer of the biofilms whereas denitrification can occur in the inner layer.

Overall, nitrification and denitrification for nitrogen removal with the UBER system could be realized simultaneously. SND was not only achieved through the whole reactor but also in the individual heterotrophic nitrification zone and hydrogenotrophic denitrification zone, respectively.

3.4. Effect of sulfate on hydrogenotrophic denitrification

As shown in Fig. 6a, when the S/N ratio was 1:2, both effluent NO₃⁻–N and SO₄²⁻–S decreased to ~5 mg L⁻¹, the concentration of XS–S gradually increased to ~8 mg L⁻¹. The average removal rate of NO₃⁻–N (1 mg (L d)⁻¹) was significantly greater than that of SO₄²⁻–S (0.44 mg (L d)⁻¹) when the effluent parameters remained stable. The concentration of NO₃⁻–N and SO₄²⁻–S kept declining when the XS–S reached about 8 mg L⁻¹. Finally, the removal rates of NO₃⁻–N and SO₄²⁻–S reached ~80%. The results indicate that effective removal of nitrate and sulfate can be achieved simultaneously at low S/N ratio since this concentration of XS–S (8 mg L⁻¹) did not inhibit hydrogenotrophic denitrification. Under a 1:1 S/N ratio, effluent SO₄²⁻–S dropped to ~10 mg L⁻¹ and XS–S went up to 15 mg L⁻¹. When the effluent concentration of NO₃⁻–N was higher than 15 mg L⁻¹ (the first 13 d), the removal rate of NO₃⁻–N (1.9 mg (L d)⁻¹) was greater than that of SO₄²⁻–S (0.54 mg (L d)⁻¹). The effluent concentration of NO₃⁻–N remained stable (7 mg L⁻¹) after 37 d, while the XS–S was 10 mg L⁻¹. At that stage, the average removal rate of SO₄²⁻–S was equal to NO₃⁻–N (1.25 mg (L d)⁻¹). After 50 d, the XS–S increased to 15 mg L⁻¹ and the SO₄²⁻–S reached a stable level (10 mg L⁻¹) (Fig. 6b). It can be inferred that the denitrification process was inhibited when the XS–S reached 10 mg L⁻¹, and sulfate reduction was inhibited when it reached 15 mg L⁻¹.

Results were similar with a S/N ratio of 2:1 (Fig. 6c). After 28 d, the concentration of XS–S reached 10 mg L⁻¹ and effluent NO₃⁻–N was stable at about 7 mg L⁻¹. The average removal rate of NO₃⁻–N was similar to SO₄²⁻–S (0.7 mg (L d)⁻¹). When the XS–S increased to 15 mg L⁻¹ at day 45, the SO₄²⁻–S equilibrium concentration (15 mg L⁻¹) was achieved. Denitrification and sulfate reduction processes were inhibited when the XS–S reached 10 mg L⁻¹ (day 28) and 15 mg L⁻¹ (day 45), respectively. The final removal rates of NO₃⁻–N and SO₄²⁻–S were below 68%. In the three groups of experiments, the denitrification percentage declined and time for stable effluent NO₃⁻–N shortened as S/N ratio increased. Further studies are needed on how sulfate inhibits hydrogenotrophic denitrification: competition for electronic donors or the toxicity of sulfide on denitrifying bacteria.

Fig. 5. DO distribution in biofilms of the heterotrophic nitrification zone (a) and hydrogenotrophic denitrification zone (b) in four phases.
4. Conclusions

The SND could be achieved with the novel UBER system for synthetic wastewater treatment. DO in bulk solution was an important factor that affected the nitrification and denitrification processes in both heterotrophic nitrification and hydrogenotrophic denitrification sections of the reactor. The experimental results indicated that high nitrogen removal efficiency could be achieved through SND by the UBER system. Relatively high DO concentration did not inhibit hydrogen autotrophic denitrification significantly. Simultaneous removal of NO$_3^-$--N and SO$_4^{2-}$--S can be achieved at low S/N ratio, but higher ratios caused inhibition of denitrification and sulfate reduction.

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